Exploration and assessment on the agronomic requirement of *Taverniera abyssinica* A. Rich: a critically endangered medicinal plant of Ethiopia

ABSTRACT

We determined the distribution and abundance of *Taverniera abyssinica* A. Rich in the Shewa floristic region, Ethiopia. We also carried out a mesh-house experiment to know whether *T. abyssinica* is able to survive and grow in any soil. From the nine potential locations we made exploration, *T. abyssinca* populations were found only in the two, Lemen and Mojo. The abundance of mature individuals was estimated to be more than 600/hectare. The one-way ANOVA results indicated that soil does not have a significant ($p>0.05$) effect on seedlings survival rate. However, it was found to have significant ($p<0.05$) effects on seedlings growth, root nodulation, and root arbuscular mycorrhizal fungi colonization. Seedlings grown on the soil collected from Lemen and Mojo produced significantly ($p<0.05$) more number of leaves and grew better than those grown on Addis Ababa (where the species was never reported to grow) soil. The root fresh weight of seedlings grown on Addis Ababa soil was found to be significantly ($p<0.05$) and 38.89% and 54.17% lower than the root fresh weight of seedlings grown on Lemen and Mojo soils respectively. We report for the first time that *T. abyssinica* is N-fixer and arbuscular mycorrhizal. Seedlings grown on the Addis Ababa soil were not colonized by arbuscular mycorrhizal fungi while those grown on Lemen and Mojo soils were. Although the estimated abundance of mature *T. abyssinica* individuals was high, there is continued exploitation of the species and habitat loss is imminent. Therefore, integrated conservation program by way of ex situ conservation, in situ conservation, and cultivation should be implemented. *Taverniera abyssinica* could be cultivated in areas with leptosol and degraded vertisol soils with slightly acidic to basic pH. Arbuscular mycorrhizal fungi could play key role in the conservation and cultivation of the species.

Keywords: Analgesic property, antipyretic property, medicinal plants (MPs), leptosol, Taveriera abyssinica, vertisol

1. INTRODUCTION

From the recorded 6000 vascular plant species of Ethiopia, the 887 are medicinal plants (MPs) of which, the 2.7% are endemic to Ethiopia [1]. *Taverniera abyssinica* A. Rich is one the most valuable endemic MPs of Ethiopia [2, 3]. Owing to its traditional use to treating sudden illnesses of all sorts, *T. abyssnica* is locally named “Dingetegna” to mean “sudden remedy”. The dried slender root (or sometimes stem) is chewed and the juice swallowed or the smoke of these plant parts is inhaled to treat, among others, fever, stomach ache, Colic, and sudden illness due to what people consider “evil spirits” [3]. Analgesic and antipyretic properties of the *T. abyssinica* root extract has been proved to be significant [4]. Its potential effect against stomachache has been demonstrated [5]. It also possesses strong nematicidal and weaker cytotoxic and

antimicrobial effects [6]. The root extract was found to contain isoflavonoids [7] which may have potential anticancer effects [8].

Unsustainable harvest and habitat loss due to agricultural expansion have resulted in the significant decline of the *T. abyssinica* natural populations. Hence, *T. abyssinica* is considered to be one of the critically endangered endemic plants of Ethiopia [9]. In light of its endemism, conservation status, and current and future economic potential, *T. abyssinica* conservation should therefore be among the primary biodiversity conservation priorities in Ethiopia. However, there has been little effort of conserving the species. The current distribution and abundance of *T. abyssinica* populations is also not well known.

One of the viable mechanisms of conserving endangered or over-exploited MPs is to cultivate them [10, 11]. Likewise, *T. abyssinica* is one of MPs recommended for cultivation [2]. In the past, there have few research works relevant to the conservation and cultivation of *T. abyssinica*. The seeds of *T. abyssinica* were determined to be orthodox and mechanical or chemical (98% sulfuric acid) treatments were known to be effective methods to break seed dormancy [12]. Effective in-virto propagation protocols of the species have also been developed [13, 14]. However, the agronomic requirement of the species is not known. Therefore, the objectives of this study were to: 1) explore for the natural populations of *T. abyssinica* in the Shewa floristic region and 2) to carryout preliminary assessment on the agronomic requirement of the species.

2. MATERIAL AND METHODS

2.1. Description of *Taverniera abyssinica*

Taverniera abyssinica A. Rich (Fabaceae/Papilionoideae) is a single foliolate or very rarely, pinnately 3-foliolate shrub or shrublet reaching up to 2 m in height. Leaflets are glabrous above while appressed-pubescent beneath. Racemes 2-8-flowered; rhachis and peduncle together c 3-25 mm long. Calyx apressed-pubescent outside; lobes equaling or longer than tube. Corolla 12-17 mm long, dark pink to purplish red. Pods are stipitate (stalk bearing), 1-3 segmented, finely pubescent and spiny (c 1.5 mm long). Young stems are covered with densely appressed-fine hairs [15, Fig. 1].

Figure 1: *Taverniera abyssinica* A. Rich. (Photo by Fisseha Asmelash).
The flower of *T. abyssinica* is Papilionaceous with diadelpous stamen.

2.2. Exploration of *Taverniera abyssinica* natural populations

Based on the Flora of Ethiopia and Eritrea [15], herbarium records, and interview with *Dingetegna* vendors in Merkato (the biggest market in Addis Ababa and Ethiopia), we determined that *T. abyssinica* populations were found in Shewa and Tigray floristic regions of Ethiopia. Hence, to assess the current conservation status of *T. abyssinica*, we made explorations in the Shewa floristic region; particularly in the localities of Adadi Mariam, Aliyu Amba, Butajira, Debralibanos, Deneba, Ensaro, Gohatsion, Lemen, and Mojo.

At each locality, relevant government offices and experts were contacted and together, local key informants (farmers and traditional healers) were identified for interview. The species was described to the key informants by also telling them the local name and showing an illustration of the species based on [15]. When key informants say they recognize the species, they were asked to locate *T. abyssinica* populations. For those places where the key informants where able to locate *T. abyssinica* populations, the name of the locality, geographic coordinate, altitude, and *T. abyssinica* abundance were recorded. Abundance was determined by counting *T. abyssinica* individuals in 30 by 30 plots laid at each site where a *T. abyssinica* population was located.

2.3. Assessment on the agronomic requirement of *Taverniera abyssinica*

2.3.1. Mesh-house experiment design

To know if *T. abyssinica* can survive and grow on soils other than where its natural populations are found, a mesh-house experiment was carried out in the Ethiopian Biodiversity Institute, Shewa floristic region, Addis Ababa. Based on the *T. abyssinica* exploration result (Table 2), Lemen and Mojo were identified to be the locations where *T. abyssinica* natural populations are found. Hence, soil from Lemen and Mojo was collected for the mesh-house experiment. Another soil from Addis Ababa where no *T. abyssinica* population has ever been reported was also collected. After four months of seedlings growth, survival rate, growth, and root traits (nodulation and mycorrhization) of *T. abyssinica* were compared. Hence, a preliminary assessment on the agronomic requirement of the species was made.

Seeds collected from the Mojo population were germinated on filter paper according to [12]. Germinated seedlings were then transplanted on eighteen 1-liter plastic pots filled with the Addis Ababa, Lemen, and Mojo soil (six pots each). On each of the pot, two *T. abyssinica* seedlings were transplanted making the total number of seedlings in the experiment 36. Hence, in this experiment, the treatment was soil (Table 1) and the three soil types were arranged in a completely random design. The experiment lasted for four months from March 31, 2022 to July 31, 2022.

Table 1: Soil type and physiochemical property of the potting soil used
(Based on World Soil Information Service data base available online: https://soilgrids.org/)

Soil characteristics	**Potting soil type**		
	Addis Ababa (9.04N,38.814E)	**Lemen**	**Mojo**
Soil type	Luvisol	Vertisol	Vertisol
Bulk density (cg/cm3)	125	128	132
Sand content (g/kg)	98	208	219
Silt content (g/kg)	516	307	401
Clay content (g/kg)	388	490	385
pH	6.1	6.7	7.1
Organic carbon (dg/kg)	271	188	174
Total nitrogen(cg/kg)	207	164	162
CEC (mmol(c)/kg	309	397	513

Note: soil property is for the 5-15cm of soil depth. Geographic coordinates for Lemen and Mojo are not provided not to expose the species for exploitation.

2.3.2. Growth and root traits measurement

After four months of growth, seedlings survival rate and growth, viz., leaf number, shoot height, shoot fresh weight, and root fresh weight were determined. Survival was computed per pot as (number of living individuals/ total number transplanted)*100. Leaf number was determined by counting leaves per plant. Shoot height was measured by a ruler while shoot fresh weight and root fresh weight were measured by analytical balance. Since, part of the root was delicate, some fine root have remained attached to the soil. Hence, the root fresh weight measured was mainly on the root part that was effectively pulled out from the soil matrix and which comprises the major proportion of the root. Two of the most

important root traits (nodulation and mycorrhizal association) were also determined by counting the number of root nodules and by measuring root arbuscular mycorrhizal fungi (AMF) colonization. Since nodule size is an important predictor of N-fixation potential [16], we counted the nodules that were easily visible with the necked eyes. Very small nodules that were not developed well were not counted. Root AMF colonization was determined by the gridline intersection method on 100 intersection points [17] by using a NOVEX light stereomicroscope (45x). Roots were first cleared in 10% KOH [18] and stained and de-stained by the ink and vinegar technique [19] using black Hero ink as stain [20].

2.3. Data analysis

One-way ANOVA was computed to know the soil preference of *Taverniera abyssinica*. The data were first checked for the equality of variances and parametric ANOVA or Kruskal–Wallis test were computed to know the effect of soil. When significant effect ($p<0.05$) was found, means were computed using Tukey honestly significant difference (HSD) or Dunn–Bonferroni tests ($p<0.05$) respectively for the parametric ANOVA or the Kruskal–Wallis test. All the statistical analysis was carried out using the R software version 4.1.1.

3. RESULTS

3.1. The conservation status of *Taveriera abyssinica*

From the nine locations where exploration was carried out, *Taverniera abyssinica* populations were found only in Lemen and Mojo (Table 2). In Mojo, three populations were located and *T. abyssinica* grows abundantly (more than 500/hectare). In Lemen also, three populations were located. However, the *T. abyssinica* abundance was much lower than Mojo (more than 70/hectare). The *T. abyssinica* populations were found in highly degraded sites with heavy clay soil. In Lemen, the species is mostly found growing solitary. However, in Mojo area, it was found growing in thickets with different plant species (Fig. 2).

Figure 2: *Taverneria abyssinica* grows in highly degraded sites; *Acacaia saligna* planted to reclaim the site is visible (A) and it can grow together with other plant species forming a thicket. Those plants with flowers (B) are *T. abyssinica* individuals.

Table 2: The location and abundance of *Taverniera abyssinica* populations in parts of the Shewa floristic region

Location	Administrative Region	Zone	*Taverniera abyssinica* presence and estimated abundance	
			Presence	Abundance
Adadi Mariam	Oromia	South West Shewa	No	Nil[§]
Aliyu Amba	Amhara	North Shewa	No	Nil
Butajira	South	Butajira	No	Nil
Debralibanos	Oromia	North Shewa	No	Nil
Deneba	Amhara	North Shewa	No	Nil
Ensaro	Amhara	North Shewa	No	Nil
Gohatsion	Oromia	North Shewa	No	Nil
Lemen	Oromia	South West Shewa	Yes	78/hectare
Mojo	Oromia	East Shewa	Yes	556/hectare

[§]local informants described the species correctly and insisted the species is present; however they were not able to locate a single site where *Taverniera abyssinica* is growing.

3.2. The agronomic requirement of *Taverniera abyssinica*

Generally, seedlings grew better on the Mojo and Lemen soil and in a descending order while they grew poorly on the Addis Ababa soil (Fig. 3). After four months of growth, the survival rate of *T. abyssinica* seedlings was 100%, 92%, and 83%, respectively for Mojo, Lemen, and Addis Ababa soils. According to the one-way ANOVA result, soil type did not have a significant ($p>0.05$) effect on survival rate (Table 3). However, soil type had significant ($p<0.05$) effects on all the remaining seedling variables measured, viz., leaf number, shoot height, shoot fresh weight, root fresh weight, root nodule number, and root AMF colonization (Table 3). The seedlings grown on the Mojo soil produced the highest leaf number which was significantly ($p<0.05$) and 23.48% and 305.71% more than the mean leaf number of the seedlings grown on the Lemen and Addis Ababa soils respectively. Similarly, the highest shoot height, shoot fresh weight, and root fresh weight was also recorded for seedlings grown on the Mojo soil to be followed by the seedlings grown on the Lemen and Addis Ababa soils respectively. Root fresh weight of seedlings grown on Addis Ababa soil was significantly ($p<0.05$) and 38.89% and 54.17% lower than the root fresh weight of seedlings grown on Lemen and Mojo soils respectively. Whereas the highest nodule number was recorded for seedlings grown on the Lemen soil, it was for the seedlings grown on Mojo soil that the highest root AMF colonization (RC) was recorded. The nodule number of seedlings on the Lemen soil was significantly ($p<0.05$) and 236.64% and 1233.33% greater than the mean nodule number of seedlings grown on Mojo and Addis Ababa soils respectively. The RC of seedlings on the Mojo soil was not significantly ($p>0.05$) greater than the mean RC of the seedlings grown on the Lemen soil. The RC of seedlings gown on both Mojo and Lemen soil were however, significantly ($p<0.05$) greater than the mean RC of seedlings grown on the Addis Ababa soil. The roots of the seedlings grown on the Addis Ababa soil were not colonized by AMF (Table 3). Root nodules recorded were mostly oval but there were also elongate/cylindrical and spherical/globose nodules. In the case of RC Vesicles were the frequently observed structures (Fig. 4).

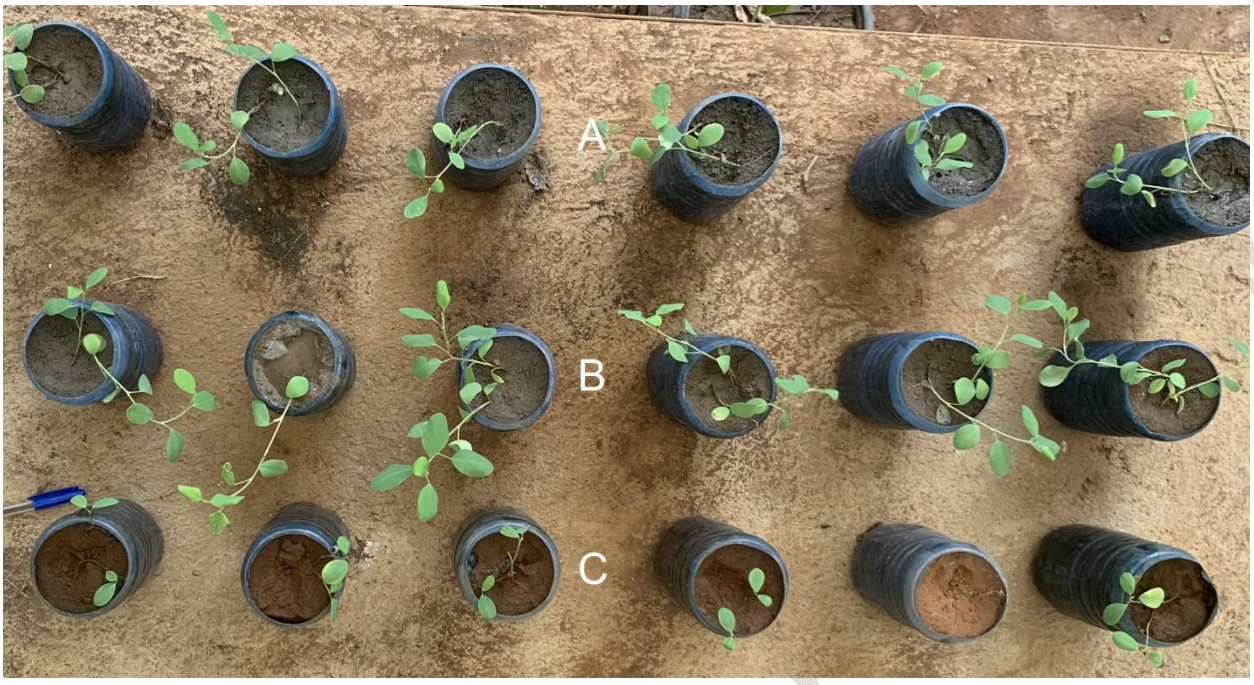

Figure 3: *Taverniera abyssinica* seedlings after four months of growth in a mesh-house in the Ethiopian Biodiversity Institute.
Row A=Lemen soil, Row B=Mojo soil, and Row C=Addis Ababa soil. Seedlings seem to grow poorly on the Addis Ababa soil.

Table 3: One-way ANOVA results and mean comparison

	Mean (±SE) values on the different soil			ANOVA	
Variables	**Addis Ababa**	**Lemen**	**Mojo**	**F**	**Chi.sq.**
Survival rate (%)	83.0(±17) ^{ns}	100.0(±0.0) ^{ns}	92.0(±8) ^{ns}	0.6	-
Leaf number	1.75(±0.25) ^{c}	5.75(±0.39) ^{b}	7.1(±0.82) ^{a}	-	24.312***
Shoot height (cm)	5.77(±0.31) ^{c}	9.44(±0.5) ^{b}	12.25(±0.9) ^{a}	25.421***	-
Shoot fresh weight (g)	0.15(±0.01) ^{c}	0.43(±0.04) ^{b}	0.65(±0.06) ^{a}	32.617***	-
Root fresh weight (g)	0.11(±0.01) ^{b}	0.18(±0.02) ^{a}	0.24(±0.04) ^{a}	5.5685*	-
Root nodule number	0.6(±0.6) ^{b}	8.0(±1.1) ^{a}	2.2(±1.0) ^{b}	17.796***	-
Root AMF colonization (%)	0.0((±0.0) ^{b}	26.17(±7.1) ^{a}	45.0(±13.3) ^{a}	-	10.753**

*significant at p<0.05, ** significant at p<0.01, and *** significant at p<0.001. Means labeled with different letters across columns are significantly different for Tukey HSD or Dunn-Bonferroni testes (p<0.05), “ns” indicates means are not significantly different (p>0.05).

Figure 4: Nodulated roots of *Taverniera abyssinica* seedlings above and arbuscular mycorrhizal fungi (AMF) colonization below.
E=elongate nodule, O=oval nodule, S=spherical nodule, V= vesicles (root colonized with AMF), NC=root not colonized with AMF. Photos are not with scale.

4. DISCUSSION

Taverniera abyssinica was categorized to be an IUCN "critically endangered" species not based on abundance of mature individuals but by its distribution, experts' judgment of population decline, and actual or potential levels of exploitation [9]. Here, we have determined an estimated abundance of *T. abyssinica* mature individuals (250-1000) which if used alone, will put the species under the "vulnerable" category. Since we found *T. abyssinica* populations in two locations (six sites), one of the criteria, i.e., existing only at a single location, that was used previously to designate the species as critically endangered [9] is also not valid currently. This could be more so if the number of mature individuals were to be accounted throughout Ethiopia (Table 4). However, we observed the species is still under exploitation for its root and in Adadi Mariam, we noticed its habitat has been changed to cultivated land. The Lemen populations were also found interspersed

within agricultural lands, which indicate agricultural expansion in to the specie's habitat have taken place. Moreover, we have observed frequent uprooting of the species particularly in the Lemen area. Hence, although the number of mature individuals could potentially be significantly high, considering the current and potential future exploitation and considering the high risk of habitat loss, the current critically endangered designation could still be appropriate. In Mojo area, we observed some of the *T. abyssinica* populations growing in thickets. This corroborates the flora record that described the species as a bush land species [15]. Therefore, *T. abyssinica* could be conserved in situ in protected bushlands/forests.

Available data indicate that *T. abyssinica* grows on leptosols and vetrisols (Table 4). Our results corroborate this data. Hence, in the mesh-house condition in the Ethiopian Biodiversity Institute (2423 m altitude), *T. abyssinica* although survived well, it did not grow fit on the Luvisol compared to the vertisol. Hence, soil type could be an important agronomic requirement of *T. abyssinica.* Leptosols and vertisols could have better CEC and copper content compared to Luvisols [21]. Hence, soil type in general and CEC and essential nutrients such as copper in particular, could be important factors to determine *T. abyssinica* growth. The soil pH could be another important factor. Seedlings grew better on the Mojo soil with a slightly basic pH compared to the Lemen and Addis Ababa soils with slightly acidic and acidic pH values. This may indicate that the species prefers slightly basic soils than acidic ones. The comparative better growth of *T. abyssinica* seedlings on the Mojo soil could also be due to home soil advantage. This is because, the seeds used in this study were collected from the Mojo populations.

Table 4: Soil type and pH of sites where herbarium record *Taverneria abyssinica* was found (Based on https://soilgrids.org/).

No	Location	Floristic region	Soil type	pH
1	Mekelle city	Tigray	Leptosol	7.9
2	Adigudem-B	Tigray	Vertisol	7.8
3	Adigudem-A	Tigray	Vertisol	7.7
4	Hagereselam	Tigray	Leptosol	7.7
5	Adi Amedy	Tigray	Leptosol	7.7
6	Mekelle/Giba plane	Tigray	Leptosol	7.5
7	Gijet	Tigray	Leptosol	7.3
8	Abala	Tigray	Leptosol	7.2
9	Gebraguracha	Shewa	Leptosol	6.4

Note: Geographic coordinates are not provided not to expose the species for exploitation. Exploration to all these locations was not possible due to security reasons

Leptosiols are problematic soils for crops growth [22]. *Taverniera abyssinica* thrives well on such soils and degraded verisols. Hence, the species must have effective soil resource acquisition mechanisms. Therefore, the assessment we made on its root traits, i.e., nodulation and mycorrhization, was important to better understand its agronomic requirement. Particularly, understanding its association with arbuscular mycorrhizal fungi (AMF) is very important [23, 24]. Based on

our results, we report for the first time that *T. abyssinica* is N-fixer at least at the seedling stage. Mature individuals in the filed were observed to be without nodules. We also report for the first time that it is arbuscular mycorrhizal. Not all legumes/Papilionoideae nodulate and are N-fixer [25]. Previous reports have indicated that the roots of *T. abyssinica* can produce isoflavonoides [7], biochemicals required for nodulation and typical of the Papilionoideae subfamily [26] Hence, our result is aligned with previous reports. The nodule number we record for seedlings grown on Mojo was not significantly (p<0.05) greater than the nodule number recorded for seedlings grown on Addis Ababa soil. However, seedlings grown on the Addis Ababa soil were not colonized by AMF. The Addis Ababa soil was stored at room temperature for more than a year while the Lemen and Mojo soils were stored only for two months. Hence, no AMF colonization could be due to the decline of infective AMF communities [27]. It could also be due to the fact that *T. abyssinica* forms association with selected AMF communities that are naturally lacking in the Addis Ababa soil [28]. The significantly (p<0.05) higher growth recorded for *T. abyssinica* seedlings grown on the Lemen and Mojo soils compared to the Addis Ababa soil could be due to AMF effect. Rhizobia (nodulation) could have no or less significant role in this regard.

5. CONCLUSION

One of our objectives was to determine the distribution and abundance of *T. abyysinica* (a critically endangered plant species) in the Shewa floristic region, Ethiopia. The other objective was to assess the agronomic requirement of the species. We have fulfilled both our objectives. From the nine locations where explorations were carried out, *T. abyssinica* populations were found only in the two, viz., Lemen and Mojo. The abundance of *T. abyssinica* was much greater than what we initially expected. However, the treat levels that were primarily used to designate its conservation status seem to persist. Hence, to improve the conservation status of the species, integrated conservation program by way of ex situ conservation, in situ conservation, and most importantly, cultivation is crucial. Based on the mesh-house experiment results, and relevant data we gathered, *T. abyssinica* could be cultivated in sites with leptosol and degraded vertisol soils with slightly acidic to basic pH. We report for the first time that *T. abyssinica* is N-fixing and arbuscular mycorrhizal. Arbuscular mycorrhizal fungi (AMF) could play key role in the future conservation/cultivation program of the species. Hence, the AMF responsiveness of *T. abyssinica* should be investigated and AMF communities associated with *T. abyssinica* should also be identified.

COMPETING INTERESTS

Authors declare no competing interest.

AUTHORS' CONTRIBUTIONS

FA conceptualized, designed the study, and produced the draft manuscript. HA and SW participated in data collection.

REFERENCES

1. EBI [Ethiopian Biodiversity Institute] (2015). National Biodiversity Strategy and Action Plan, Addis Ababa, Ethiopia.
2. Bekele, E. (2007). Study on Actual Situation of Medicinal Plants in Ethiopia. available on line: http://jaicaf.or.jp/publications/ethiopia_ac.pdf
3. Kloos, H., Menberu, T., Tadele, A., Chanie, T., Debebe, Y., Abebe, A., Zealiyas, K., Tadele, G., Mohammed, M., and Debella, A. (2014). Traditional medicines sold by vendors in Merkato, Addis Ababa: Aspects of their utilization, trade, and changes between 1973 and 2014. Ethiop. J. Health Dev., 28(2):1-17
4. Dagne, E., Yenesew, A., Capiso, F., Mascolo, N., and Pinto, A. (1990). Preliminary studies on antipyretic and analgesic properties of Taverniera abyssinica. Ethiop. Med. J., 28:155-162.
5. Noamesi, B. K. and Dagne, E. (1990). Intestinal smooth muscle spasmolytic actions of the aqueous extracts of Taverniera abyssinica. J. Ethnopharm., 30:71-81.
6. Stadler, M., Dagne, E., and Anke, H. (1994). Nematicidal activities of two phytoalexins from Taverniera abyssinica. Planta Med., 60(6):550-2.
7. Duddeck, H., Yenesew, A., and Dagne, E. (1987). Isoflavonoids from Taverniera abyssinica, Bull. Chem. Soc. Ethiop., 1: 36-41.
8. Militao, G.C.G., Dantas, I.N.F., Pessoa, C., Falcao, M.J.C., Silveira, E.R., Lima, M.A.S., Curi, R., Lima, T., Moraes, M.O., and Costa-Lotufo, L.V. (2006). Induction of apoptosis by pterocarpans from Platymiscium floribundum in HL-60 human leukemia cells. Life Sci., 78(20): 2409-2417.
9. Vivero, J.L., Kelbessa, E., and Demissew, S. (2005). The Red List of Endemic Trees & Shrubs of Ethiopia and Eritrea. Fauna & Flora International,Cambridge, UK. pp. 23
10. FAO, FLD, and IPGRI (2004). Forest genetic resources conservation and management. Vol. 1: Overview, concepts and some systematic approaches. International Plant Genetic Resources Institute, Rome, Italy.
11. WHO, IUCN, and WWF (1986). Guidelines on the conservation of medicinal plants. World Health Organization (WHO), Geneva, Switzerland, and WWF –World Wide Fund for Nature, Gland, Switzerland. 38p
12. Addis, G. (2003). Treatments promoting germination of Taverniera abyssinca A. Rich. Seeds. Seed Sci. & Technol.; 31:579-586.
13. Gelan, B. (2015). In Vitro Propagation of Taverniera abyssinica A. Rich (Dingetegna). MSc Thesis. Institute of Biotecnology, Addis Ababa University. 39p
14. Abera, B., Negash, L., Kumlehn, J., & Feyissa, T. (2010). In vitro regeneration of Taverniera abyssinica A. Rich: a threatened medicinal plant. Ethiopian Journal of Education and Sciences, 6(1):59-71
15. Thulin, M. (1989). Papilionoideae. In: Flora of Ethiopia, Volume 3. pp. 97-25. (I. Hedberg and S. Edwards, eds.). Addis Ababa University and Uppsala University
16. Brockwell, J., Searle, S.D., Jeavons, A.C., Waayers, M. (2005). Nitrogen fixation in acacias: an untapped resource for sustainable plantations, farm forestry and land reclamation. ACIAR Monograph No. 115. 132p.
17. Giovannetti M, Mosse B. (1980).An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500
18. Brundrett M., Bougher N., Dell B., Grove 359 T., Malajczuk N. (1996). Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32. Australian Centre for International Agricultural Research, Canberra, p 374
19. Vierheilig H, Coughlan AP, Wyss U, Piche Y. (1998). Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microb 64:5004–5007

20. Asmelash, F., Bekele, T., Kebede, F., Belay, Z. (2021). The arbuscular mycorrhizal fungi status of selected tree nurseries in the Ethiopian highlands. Journal of Forestry Research 32 (3): 1189-1201. doi: 10.1007/s11676-020-01169-9
21. Elias, E. (2019). Selected chemical properties of agricultural soils in the Ethiopian highlands: a rapid assessment. South African Journal of Plant and Soil, 36(2), 153-156.
22. Nyssen, J., Tielens, S., Gebreyohannes, T., Araya, T., Teka, K., et al. (2019). Understanding spatial patterns of soils for sustainable agriculture in northern Ethiopia's tropical mountains. Plos one, 14(10), e0224041.
23. Koziol, L., Schultz, P. A., House, G. L., Bauer, J. T., Middleton, E. L., & Bever, J. D. (2018). The plant microbiome and native plant restoration: the example of native mycorrhizal fungi. BioScience, 68(12), 996-1006.
24. Asmelash, F., Bekele, T. and Birhane, E., (2016). The Potential Role of Arbuscular Mycorrhizal Fungi in the Restoration of Degraded Lands. Frontiers in Microbiology 7:1095: 1-15.
25. Corby, H. D., Smith, D. L., and Sprent, J. I. (2011). Size, structure and nitrogen content of seeds of Fabaceae in relation to nodulation. Botanical Journal of the Linnean Society, 167(3), 251-280.
26. Liu, C-W. and Murray, J.D. (2016).The Role of Flavonoids in Nodulation Host-Range Specificity: An Update. Plants, 5 (33):1-13
27. Varga, S., Finozzi, C., Vestberg, M., and Kytöviita, M. M. (2015). Arctic arbuscular mycorrhizal spore community and viability after storage in cold conditions. Mycorrhiza, 25(5), 335-343.
28. Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology, 84(9), 2292-2301